7. A. E. Wood, *J. Paleontol.* **23**, 556 (1949); L. Radinsky, *Yale Peabody Museum Bull.* **17** (1963).
8. The Carnegie Museum's portion of this study is supported in part by NSF grant GB 1266; the University of Colorado Council on Research and Creative Work supported the University Museum's field work and illustrations. We thank M. C. McKenna of the American Museum of Natural History for advice and criticism.

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Radiocarbon Dating of a Late Paleolithic Culture from Egypt

Abstract. *Two radiocarbon dates of about 12,000 B.C. for a new prehistoric culture from a stratified site at Kom Ombo, Upper Egypt, throw light on a deposition phase of the late Pleistocene Nile. The dates reveal that the associated blade industry is coeval with at least the later part of the Upper Paleolithic in Europe and Southwestern Asia.*

Evidence for the existence of Paleolithic man in Egypt has been known for almost a century, and during that time large numbers of surface collections of artifacts have been made and a relatively small number of sites excavated. To my knowledge, however, no radiocarbon date for a Paleolithic culture has yet been published, although some dates have been obtained from Dynastic, Predynastic, and Neolithic materials (*1*). This lack is all the more marked when contrasted with the situation in other parts of North Africa (particularly Libya) and in southwestern Asia where a number of Paleolithic dates have been obtained in the past decade. It reflects not only the infrequency of sealed sites such as caves or rock-shelters but also the near cessation of prehistoric research in Egypt since the second World War.

It seems worthwhile, therefore, to record two radiocarbon dates recently received in connection with a newly discovered culture of the late Paleolithic in Upper Egypt. During 1962 to 1963 the National Museum of Canada sponsored a prehistoric archeological expedition to Egypt to take part in the current international Aswan Reservoir salvage program. The University of Toronto also collaborated in this research, and I served as director of the expedition. Since a large area of desert was being reclaimed at Kom Ombo, about 45 km north of Aswan, in order to resettle the larger part of the population being displaced from Egyptian Nubia by the rising waters behind the new High Dam, the Canadian expedition concentrated its efforts here where late Paleolithic sites had been reported many years ago. (*2*).

A large number of sites was found in and on the silts deposited by the late Pleistocene Nile, and a series of late Paleolithic cultures not hitherto reported from the Nile Valley was identified. One of these was recovered from a stratified occupation site buried in the silts about 3 km east of the present Nile near Gebel Silsilah. Accompanying large quantities of faunal remains were flint artifacts characterized mainly by retouched blades and bladelets and by occasional burins and scrapers. The nuclei are usually long and prismatic with plain oblique striking platforms. No geometric microliths or microburins were found in this industry, and there was no evidence of pottery, polished stone, or food production. The name *Sebekian* (after Sebek, one of the patron deities of Kom Ombo in Pharaonic times) has been given to this new culture, for which a full report is being prepared.

From two specimens of charcoal recovered near hearth areas in two separate parts of the site the following ages have been calculated (*3*):

I-1291	14,240 ± 370 ago (12,290 B.C.)
I-1292	14,100 ± 450 ago (12,150 B.C.)

These ages are not only highly consistent with each other but also agree well with local geological evidence. The results of further samples now being run from this and other sites at Kom Ombo will shortly be made available. It is hoped that studies now under way on the faunal materials, soils, and possible paleobotanical remains from these sites will help document the climate and ecology of this part of the Nile Valley during this phase of human occupation. The aforementioned dates not only show that the Sebekian culture was coeval with Upper Paleolithic cultures in such regions as Western Europe (for example the Middle Magdalenian) and southwestern Asia; they also provide relative ages for several other different lithic industries with which the Sebekian is in stratigraphic relationship at Kom Ombo. In addition, we now have a geologically useful absolute dating for the period when the late Pleistocene Nile was still depositing silts in what is now desert before it shrank into its modern narrow floodplain during Holocene times. This should supplement the data on Nile geological history recently presented by Fairbridge (*4*) and other data which may be expected soon from current investigations in Egyptian and Sudanese Nubia (*5*).

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References and Notes

1. For a complete listing of all archeological radiocarbon dates from Egypt up to 1961, see A. J. Jelinek, *Current Anthropol.* **3**, 451 (1962).
2. E. Vignard, *Une nouvelle Industrie lithique, le Sébilien* (Institut française d'Archéologie orientale, XXII, Cairo, 1923).
3. Isotopes, Inc.
4. R. W. Fairbridge, *Nature* **196**, 108 (1962).
5. R. Said and B. Issawy "Preliminary results of a geological expedition to Lower Nubia and to Kurkur and Dungel Oases, Egypt," *Contributions to the Prehistory of Nubia*, No. 1, F. Wendorf, Ed. (Museum of New Mexico, Santa Fe, 1964).

1 June 1964

Purified Interferons: Physical Properties and Species Specificity

Abstract. *The antiviral activity of highly purified preparations of chick and mouse interferons has marked species specificity. This species specificity is not explained by a demonstrable difference in adsorption rates. There is no difference in charge between the molecules as measured by combined zone electrophoresis or ion-exchange chromatography. The interferons are distinguishable by thermal inactivation studies and by precise chromatography on G-100 Sephadex columns. With the latter method, interferons produced by the same cell species (i) in vivo or in vitro, or (ii) in response to different viruses, have been shown to be identical. The same virus stimulates physically distinguishable molecules in the two different cell species. These findings indicate that interferon is a virus-induced product of the host genome.*

The molecular weight and charge of chick interferon have been disputed recently (*1–4*). Crude interferons have been prepared by others (*5*) in which the antiviral activity measured in heterologous assays varied from 1 to 10 percent of the homologous antiviral effect. No precise physicochemical

comparisons have been carried out between interferons stimulated by different viruses in the same cell in vitro and in vivo or by the same virus in different cell species. Such comparisons allow clear demonstration of the role of virus and cell in interferon production. The availability of purified and concentrated chick and mouse interferons (*6*) in this laboratory has allowed direct investigation of these problems.

Chick interferon was stimulated by a WS strain of influenza virus in embryonated eggs and by Chikunguna virus in tissue culture. Mouse interferon was produced by Chikunguna virus in tissue culture and in vivo (*7*) by intravenous injection of Newcastle disease virus. Chick interferon produced in ovo and mouse interferon produced in vitro were purified by methods which include precipitation with zinc acetate (*2*) and gradient chromatography on carboxymethyl-Sephadex. Both interferons were obtained in high yield, and unit activity per milligram of protein was increased 6000-fold over starting materials (see *6*).

A plaque inhibition assay was employed with a 50 percent inhibition end-point for estimation of interferon titer. Six to 12 hours after adsorption of interferon to confluent monolayers of primary mouse or chick fibroblasts growing in tissue culture plates, the cells were challenged with vesicular stomatitis virus. The virus was allowed to adsorb from 0.5 ml of medium for 45 minutes before overlaying with nutrient agar.

The host cell specificity of the antiviral activity was evaluated by concurrent assay on homologous and heterologous cells of highly concentrated and purified chick and mouse interferons. Purified mouse interferon did not protect chick cells; purified chick interferon did not protect mouse cells. Mouse interferon with a titer of 1/20,000 on mouse cells failed to protect chick cells at a dilution of 1/30. Chick interferon with a titer of 1/4000 on chick cells demonstrated no protection on mouse cells at 1/10.

Thus, not even 0.1 percent of the antiviral effect shown in the homologous system could be demonstrated in the heterologous system. This species specific character of the assay system permitted precise physical comparison, as one interferon could be measured in the presence of the other.

To determine whether host cell specificity was due to a difference in adsorption rates, 4-ml samples of purified chick or purified mouse interferon were allowed to adsorb on confluent monolayers either of chick or mouse cells at 38°C for 0, 1, 3, 5, and 7 hours. The fluids were sampled at each time interval and assayed for residual interferon. Both interferons were found to have similar rates of adsorption on chick cells through this period. Chick interferon also showed identical adsorption rates on chick and mouse monolayers. The rate of disappearance of interferon from these supernatants paralleled the rate of appearance of its antiviral effects in similarly treated monolayers of homologous cells. Control experiments were performed to rule out inactivation by the medium and adsorption to the container.

Both electrophoresis and chromatography were employed to compare and characterize the charge of chick and mouse interferon. Combined electrophoresis on cellulose acetate (Fig. 1) showed no difference between purified chick and mouse interferons. Combined chromatography on carboxymethyl Sephadex (Fig. 2) showed both interferons to have an identical emergence position despite a gradient with a very gradual change in *p*H.

These studies indicate indistinguishable molecular charges for chick and mouse interferons, and studies by others (*4*) have shown them to have similar molecular weights. Identical sedimentation constants have been demonstrated by Rotem and Charlewood for these interferons when both are compared to radioactive egg white lysozyme markers in a sucrose density gradient ultracentrifugation (*4*).

Previous work with crude materials suggested a difference in thermal inactivation of chick and mouse interferons, but it was recognized that other factors in the impure preparation could have produced this difference (*7*). To investigate this question, samples of purified interferon were incubated for 1 hour at several temperatures, between 4° and 80°C. Purified mouse interferon was found to be more heat labile than purified chick interferon (Fig. 3). Although the thermal inactivation differences reflected a difference in structure, the intrinsic error and the replicate variability of the interferon assay limits

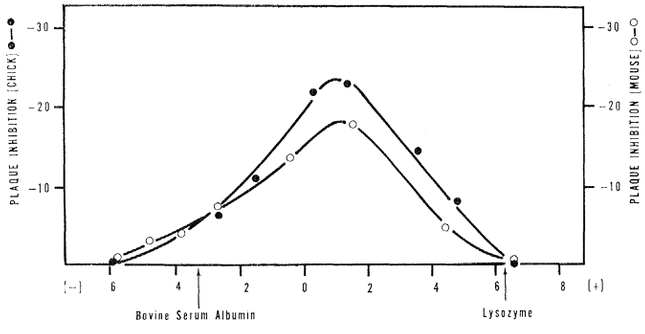
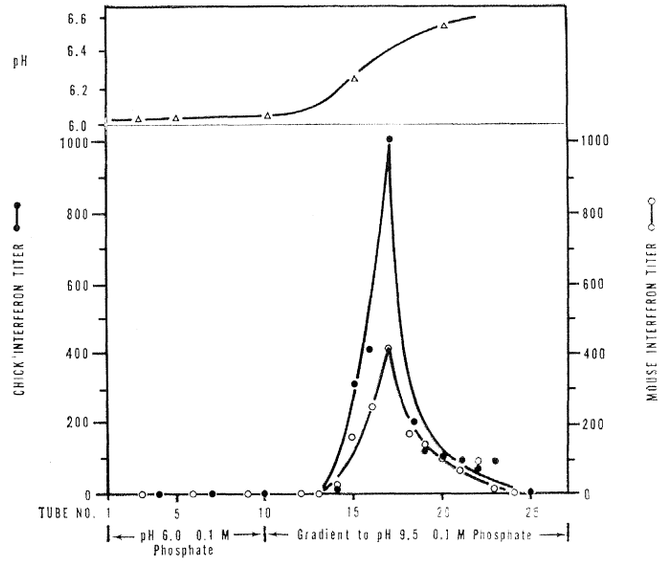
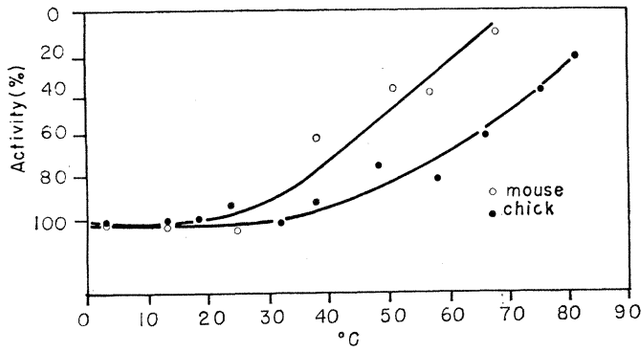


Fig. 1 (above). Electrophoresis of combined mouse and chick interferons on cellulose acetate strips at *p*H 8.6 in 0.1*M* tris buffer (*2*). Bovine serum albumin and lysozyme were used as markers. Strip sections were eluted with 0.05 percent bovine serum albumin in tissue culture media for assay. Fig. 2 (right). Chromatography of combined mouse and chick interferons carried out at 4°C on a 15- by 1-cm column of carboxymethyl-Sephadex which was developed with a 0.1*M* sodium phosphate *p*H gradient.





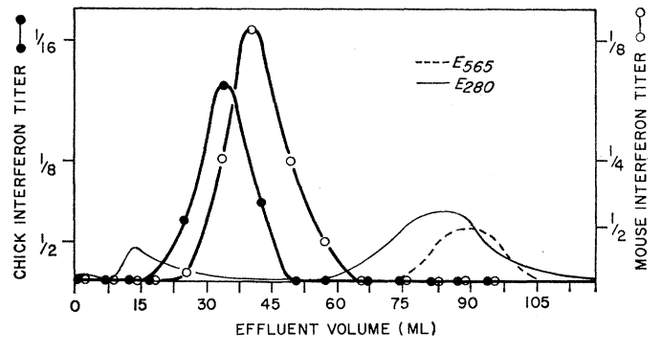


Fig. 3 (left). Heat inactivation of purified interferon. Activity was measured after 1-hour incubation periods at indicated temperatures in 0.1*M* sodium phosphate (*p*H 6.4). Fig. 4 (right). Chromatography of combined mouse and chick interferons carried out at 4°C in phosphate-buffered saline (*13*) on a 120- by 1.2-cm column of G-100 Sephadex. A mixture of crude chick and mouse interferons was used, each prepared in tissue culture. The first peak seen on OD_{280} measurement is due to protein and the second to amino acids in the tissue culture supernatant. The OD_{505} peak is due to phenol red present in the tissue culture media.

this approach for precise comparisons.

Combined chromatography on G-100 Sephadex was used to demonstrate a structurally dependent difference between these two interferons. Dextran gels separate molecules primarily on the basis of size. However, sorption effects occur because of charged or aromatic amino acid residues which can influence migration rates through the old gel bed (*8*). Chick and mouse interferons, whether crude or purified, could clearly be distinguished by precise gel filtration even when the stimulating virus was the same (Fig. 4). This difference in chromatographic behavior was present whether the interferons were stimulated in vivo or in vitro and depended on the cell species in which it was produced rather than the virus employed.

These studies with highly purified materials indicate that both interferons migrate cathodally at *p*H 8.6 and that both have indistinguishable charges on zone electrophoresis or on gradient ion exchange column chromatography. The findings of Lampson *et al.* (*2*) on the electrophoretic properties of chick interferon were confirmed. However, as the chick interferon adsorbed to the cellulose acetate and required bovine serum albumin for elution, it could not be used for precise determination of the isoelectric point.

A degree of purification and concentration higher than attained previously has also allowed demonstration of host cell specificity of action and the similarity of interferon adsorption of homologous and heterologous cells. Independent concurrent studies with highly active crude interferons have confirmed the strict host cell specificity (*9*). The reported heterologous antiviral activity (*3, 5, 10*) of mouse and chick interferons and perhaps interferons from other species should now be reexamined. These activities might be due to residual interfering virus or nonspecific antiviral factors derived from cells. Crude interferons which exhibited some activity on heterologous cells have been reported to show small differences in adsorption rates as measured on homologous and heterologous cells (*5*).

The data of the G-100 Sephadex experiment were compared with those of experiments with marker protein of known molecular weights, and values for chick and mouse interferons were determined to be approximately 38,000 and 26,000, respectively. Results which confirm ours were obtained recently by gel filtration of crude chick interferon (*3*). These values differ only slightly from the molecular weight calculations based on density gradient ultracentrifugation. In one such study, the molecular weights of both interferons appeared to be between 19,000 and 23,000 (*4*), while Lampson *et al.* (*2*) found the molecular weight of chick interferon to be between 20,000 and 34,000.

The question of the primary binding site and mechanism of action of interferon is still open. Preparation of purified radioactive interferon of high specific activity should allow autoradiographic demonstration of its site of action. Gladsky and Holper (*11*) have demonstrated that crude interferon of the homologous cell species inhibits the RNA synthesizing activity of extracts of mammalian cells infected with virus. This result is compatible with our studies and indicates that species specificity of interferon is not mediated by differences in adsorption. However, other studies (*12*) have suggested that the viral associated polymerase is coded on the viral genome. Hence it is puzzling how interferon can effect a host specific inhibition of this enzyme.

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References and Notes

1. D. C. Burke, *Biochem. J.* **78**, 556 (1961); A. Davies, *ibid.* **90**, 29 (1964); L. E. Kreuz and A. H. Levy, *Nature* **200**, 883 (1963).
2. G. P. Lampson, A. A. Tytell, M. M. Nemes, M. R. Hilleman, *Proc. Soc. Exptl. Biol. Med.* **112**, 468 (1963).
3. A. W. Phillips and R. D. Wood, *Nature* **201**, 819 (1964); C. Jungwirth and G. Bodo, *Biochem. Z.* **339**, 382 (1964).
4. Z. Rotem and P. A. Charlewood, *Nature* **198**, 1066 (1963).
5. G. E. Gifford, *J. Gen. Microbiol.* **33**, 437 (1963).
6. T. C. Merigan and C. A. Winget, in preparation.
7. S. Baron and C. E. Buckler, *Science* **141**, 1061 (1963).
8. J. Porath, *Biochim. Biophys. Acta* **39**, 193 (1960).
9. S. Baron, S. Barban, C. E. Buckler, *Science*, this issue.
10. A. Isaacs, *Advan. Virus Res.* **10**, 1 (1963).
11. A. Glasky, L. Simon, J. C. Holper, *Science*, **144**, 1581 (1964).
12. A. Lwoff, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 159 (1962).
13. R. Dulbecco and M. Vogt, *J. Exptl. Med.* **99**, 167 (1954).
14. I thank Mrs. C. A. Winget for her able technical assistance, and Samuel Baron and Charles Buckler for a gift of mouse serum interferon and an introduction to the methods of interferon measurement used in these studies. Supported by USPHS grant AI-05629-01.
15. Recent work in our laboratory indicates human interferon induced in tissue culture by Newcastle disease virus also shows a strict host cell specificity. It is not active on chick or mouse cell in 1000-fold excesses nor are purified chick or mouse interferons active on human cells in similar excesses.

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